

KKpn-1 were provided by Akira Matsumoto (Okayama, Japan); C. pneumoniae strains WI-02 to WI-06 were provided by The Center of Disease Control (Atlanta, Georgia); C. pneumoniae strain UZG-1 was provided by Jacobus M. Ossewaarde (Bilthoven, The Netherlands). The respective coding region of C. pneumoniae gene BVH-CPN1 from these strains were amplified by PCR (DNA Thermal Cycler GeneAmp PCR system 2400 Perkin Elmer, San Jose, California) from genomic DNA using the following oligos: COUF29 (5'-CTCTCCGTCCATATGTGTGACGTACGGTCTAAGG-3'); (SEQ ID NO: 39) COUF31 (5'-TCAGGAGGATCCACTTACTTAGTCATTCACCTTGATTTCCTTCTTG -3') (SEQ ID NO:40). PCR products were purified from agarose gel using a QIAquick gel extraction kit from QIAGEN following the manufacturer's instructions (Chatsworth, California) and the DNA insert were sequenced (Taq Dye Deoxy Terminator Cycle Sequencing kit, ABI, Foster City, California). Length of PCR fragments generated for all these strains was identical and pairwise comparisons of the nucleotides and the predicted amino acid sequences from four of these strains (CWL-029, KKpn-1, Wi-04 and UZG-1) revealed 100% identity. (PCR fragments of other strains have not been sequenced). --

B1
concl

Please replace the paragraph on Page 39, beginning at line 11 with the following:

-- The purification of the recombinant proteins from the soluble cytoplasmic fraction of IPTG-induced AD494(DE3)pLysS/rpET19 was done by affinity chromatography based on the properties of the His•Tag sequence (10 consecutive histidine residues) (SEQ ID NO:77) to bind to divalent cations (Ni^{2+}) immobilized on the His•Bind metal chelation resin. Briefly, the pelleted cells obtained from a 500 mL culture induced with IPTG was resuspended in lysis buffer (20 mM Tris, 500 mM NaCl, 5 mM imidazole, pH 7.9) containing 1 mM of (Phenylmethylsulfonyl fluoride (PMSF; Sigma), sonicated and centrifuged at 16,000 X g for 30 min to remove debris. The supernatant was deposited on a Ni-NTA agarose column (QIAGEN, Mississauga, Ontario, Canada). The C. pneumoniae BVH-His•Tag-CPN1 recombinant protein was eluted with 500 mM imidazole-500mM NaCl-20 mM Tris pH 7.9. The removal of the salt and imidazole from the sample was done by dialysis against PBS at 4°C. The quantity of recombinant protein obtained from the soluble

B2

of core

fraction of E. coli was estimated by MicroBCA (Pierce, Rockford, Illinois). --
